Regioselective enzymatic synthesis of estradiol 17-fatty acid esters

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Dedicated to Professor Rosa M. de Lederkremer on her 70th aniversary

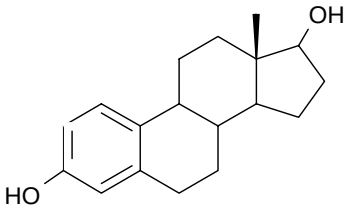
Abstract

A series of acyl esters of 3,17-β-estradiol has been prepared by an enzymatic methodology. Eleven 17-monoacyl products (five novel compounds) were obtained in a highly regioselective way by acylation of 3,17-β-estradiol or by alcoholysis of the corresponding diacyl derivatives. The influence of various reaction parameters such as molar ratio, enzyme:substrate ratio and temperature was evaluated. Among the tested lipases, *Candida rugosa* lipase appeared to be the most appropriate in monoacylation and lipase from *Candida antarctica* in alcoholysis. The advantages presented by this methodology such as mild reaction conditions, economy and low environmental impact, make the biocatalysis a convenient way to prepare monoacyl derivatives of 3,17-β-estradiol containing the aromatic 3-hydroxyl group free. Some of these compounds are recongnized as useful products in the pharmaceutical industry.

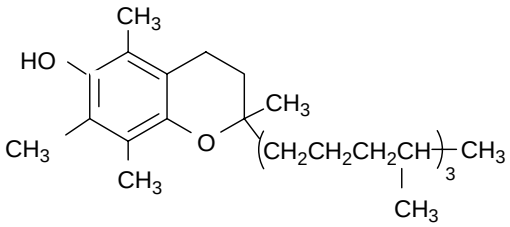
Keywords: Lipases. enzymatic acylation and alcoholysis. 3,17-β-estradiol esters

Introduction

Human estrogens, such as 3,17-β-estradiol **1,** are known to exert powerful antioxidant effects in lipid-aqueous systems in vitro.[1-3] The antioxidant efficacy appears to depend on unsubstituted hydroxyl groups on the aromatic ring A of estrogen molecules,[4-7] a structure similar to that seen in other natural antioxidants such as α-tocopherol **2** and several isoflavone phytoestrogens as well as flavones (Figure 1).[8]



1



2

Figure 1

The identification of estradiol esters formed by lecithin:cholesterol acyltransferase[9] and the esterification site of estradiol at C-17 in the D ring,[10] suggested that these derivatives might become structural components in lipoproteins. These lipoprotein-associated molecules would be active possibly interfering with the atherosclerotic process.

Fatty acid esterification of steroid hormones is a metabolic transformation which prolongs and potentiates hormonal activity, although the esters are prohormones which require hydrolytic cleavage for their activation.[11] In theory, estrogen fatty acid esters could constitute a hormonal reservoir mainly residing in fat tissue.[12] One may speculate that specific esterases might become activated and liberate the estrogens at times when steroidogenic organs become quiescent or lose their function.[11]

Although there is mounting evidence supporting important functions for steroid esters, all the functions remained speculative and further studies are of particular interest. Then, fatty acid monesters of 3,17-β-estradiol in the position 17 of ring D could be useful to ascertain the possible utility of estrogens as protective against atherosclerosis and to provide standards for metabolic studies.

The regioselective tranformation of polyfunctional compounds such as steroids, is a challenging problem in organic synthesis, especially in case of a structure sensitive to acid, base, oxidation, or reduction, which limits the choice of reagents needed to accomplish a particular transformation. In recent years, lipases have become attractive as biocatalysts for chemo-, regio- and stereoselective reactions under mild conditions.[13,14] They can be used in a wide variety of organic solvents and do not require a coenzyme for activity.[15,16]

Specifically in the steroids field, enzyme catalysis can play an important role for the mild and selective interconversion of functional groups via regioselective transformations.[17-21] Studies carried out in our laboratory on the esterification and transesterification of polyfunctionalyzed steroids, have shown that lipases can act on substituents either on A-ring or on the D-ring. [22,23] Thus, in previous papers we observed that, in androstanes and pregnanes, *Candida rugosa* lipase showed a preference for C-3 hydroxyl or acyloxy groups, whereas *Candida antarctica* catalyzed the reactions in D-ring.[24,25]

Since 17-monoacyl esters of 3,17-β-estradiol are biologically active compounds used as "long acting" estrogens, a simple and selective procedure to obtain them is required. In

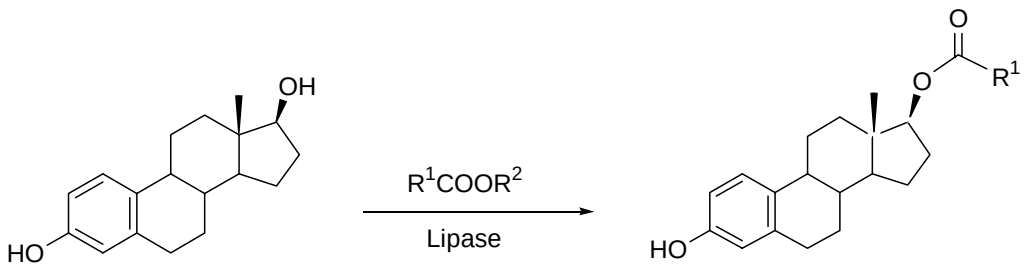
continuation of our work on enzymatic transformation of steroids, we report in the present paper the results obtained in lipase-catalyzed acylation of 3,17-β-estradiol **1** and alcoholysis of its dioleoyl derivative.

Results and Discussion

We have prepared, under mild reaction conditions monoacylated derivatives of 3,17-β-estradiol in a regioselective way and in high yields. The different compounds have been obtained through acylation of the substrate or alcoholysis of the oleyl derivative using lipases from several sources as catalysts.

1. Enzymatic acylation

The enzyme-catalyzed acylation let to obtain, in a regioselective way, monoacylated derivatives with the acyl group exclusively suited in the 17 position of ring D in the steroidal skeleton (Scheme 1).



1 **3**

3a	$R^1 = CH_3(CH_2)_3CO$-	$R^2 = CH_3CH_2$-
3a	$R^1 = CH_3(CH_2)_3CO$-	$R^2 = H$
3b	$R^1 = CH_3(CH_2)_4CO$-	$R^2 = H$
3d	$R^1 = CH_3(CH_2)_8CO$-	$R^2 = H$
3d	$R^1 = CH_3(CH_2)_{10}CO$-	$R^2 = H$
3e	$R^1 = CH_3(CH_2)_{12}CO$-	$R^2 = H$
3f	$R^1 = CH_3(CH_2)_{14}CO$-	$R^2 = H$
3g	$R^1 = CH_3(CH_2)_{16}CO$-	$R^2 = H$
3h	$R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$- *cis*	$R^2 = H$
3i	$R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$- *trans*	$R^2 = H$
3j	$R^1 = CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7CO$- *cis, cis*	$R^2 = H$
3k	$R^1 = CH_3(CH_2CH{=}CH)_3(CH_2)_7CO$- *cis, cis, cis*	$R^2 = H$

Scheme 1

1.a. Enzyme screening and solvent effect

Several commercial lipases in different solvents were evaluated in the acylation reaction of **1** with oleic acid: *Candida rugosa* lipase (CRL), *Candida antarctica* lipase B (CAL B); Lipozyme from the fungus *Rizomucor miehei* (LIP), pancreatin, porcine pancreatic lipase (PPL) and lipase from *Pseudomonas* sp. (PS-C). The solvents selected were acetone, *t*-butanol, *t*-amyl alcohol, diisopropyl ether and toluene. Reactions were carried out at 33°C using an enzyme: substrate ratio of 5 and acylating agent: estradiol ratio of 2.5. TLC monitoring allowed the identification of the lipase able to promote the monoacylation of **1**.

In the absence of biocatalyst no product was obtained and, among the tested lipases, only CRL gave satisfactory results. GC/MS analysis of the CRL-catalyzed esterification in toluene showed that this lipase was completely regioselective obtaining exclusively the oleyl derivative in 17 position **3h** in 62% yield, whereas in diisopropylether a mixture of **3h** and isopropyl oleate was obtained.

Working in the above mentioned reaction conditions, it is known that microbial lipases accept equatorial 3β-OH (PSL, CRL) or axial 3α-OH (CAL B) of ring A in the steroidal skeleton, but none of the lipases tested in this case was active in acylation of the aromatic 3-hydroxyl group in estradiol in the presence of solvents.

The application of lipases let to obtain 17-monooleyl **3h** derivative with a high degree of selectivity, achieving a remarkable improve to the chemical approach. According to literature it is not possible to obtain exclusively the product **3h**. The chemical acylation of **1** with oleyl chloride in pyridine at 0°C affords a mixture of products: a major product which is the 3,17 diester and two minor products corresponding to the C-3 monoester and C-17 monoester, as well as free estradiol.[26,27]

Considering the results of enzymatic screening, we observed that CRL was the enzyme of choice for the synthesis of 3,17-β-estradiol-17-oleate **3h** using toluene as solvent and was then selected for any further experiments.

1.b. Effect of nature of the acyl donor

Using CRL in toluene we investigated the influence of the acyl donor on the reaction yield. Estradiol esterification with oleic acid was compared to transesterification using ethyl oleate as acyl donor. We observed that esterification with oleic acid was much better with more than 75 % formation of **3h** while the use of ethyl ester gave very poor yield not exceeding 39% after 96 h. Interestingly, the same results were also reported by us in enzymatic acylation of pyridoxine[28] and dehydroepiandrosterone[23] and other authors in the preparation of cholesterol-[29] and phytosterols oleate.[30] Therefore, in all further experiments with CRL, esterification processes were chosen using free fatty acids as acyl donors.

1.c. Effect of acylating agent : substrate ratio

The effect of the influence of acylating agent: substrate ratio on reaction yield was evaluated in the esterification of estradiol **1** with oleic acid in toluene using CRL. Reaction courses were

followed by GC at 24 and 72 h of reaction. As expected, it was observed that a molar excess of fatty acid was advantageous for the reaction (Table 1) with 2.5:1 oleic acid/ estradiol ratio giving the best results (78 % esterification degree after 72 h, entry 2). A higher excess of fatty acid did not improve yields in comparison with 2.5 ratio.

Table 1. Acylating agent/substrate ratio on the CRL-catalyzed synthesis of 3,17-β-estradiol-17-oleate **3h**

Entry	A/S	Yield (%)[a]	
		24 h	72 h
1	1:1	34.6	56.7
2	2.5:1	65.7	78.3
3	5:1	62.4	73.5
4	10:1	44.4	70.2
5	20:1	38.5	63.9

[a] Determined by GC analysis. E/S: 5, temperature: 55°C.

1.d. Effect of enzyme:substrate ratio

The influence of the enzyme:substrate ratio was evaluated with a 2.5:1 estradiol/oleic acid ratio varying the amount of CAL. 3,17-β-Estradiol-17-oleate **3h** formation was then monitored at 72h by GC (Figure 2). From the obtained results, it can be concluded that a ratio E/S of 5 is the most satisfactory.

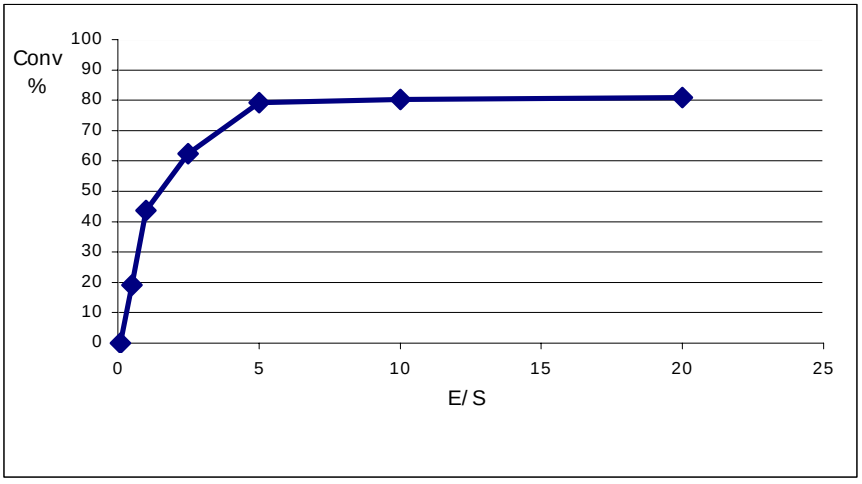


Figure 2. Effect of enzyme:substrate ratio on the CRL-catalyzed synthesis of 3,17-β-estradiol-17-oleate (**3h).** Acylating agent /S: 2.5, temperature: 55°C, time 72h.

1.e. Influence of temperature on 3,17-β-Estradiol-17-oleate formation

Temperatures of 33°C and 55°C were considered in reactions where the previously studied parameters were fixed to their optimal values (CRL, toluene, E/S: 5 and oleic acid/estradiol: 2.5). The obtained results: 62 % at 33°C and 79% at 55°C, showed a difference in terms of yields for the catalyzed reaction at both temperatures. So we decided to perform the reaction at 55°C.

1.f. Application of the enzymatic acylation to several acylating agents

The results obtained by means of the CRL catalyzed procedure to the preparation of 17-monoacyl fatty acid derivatives of 3,17-β-estradiol are shown in Table 2. Variable chain length saturated and unsaturated carboxylic acids from 5 to 18 carbon atoms gave the monoacyl derivative exclusively in position 17 in high yield. The results show that the ester formation was not influenced by chain length nor by the presence or stereochemistry of double bonds.

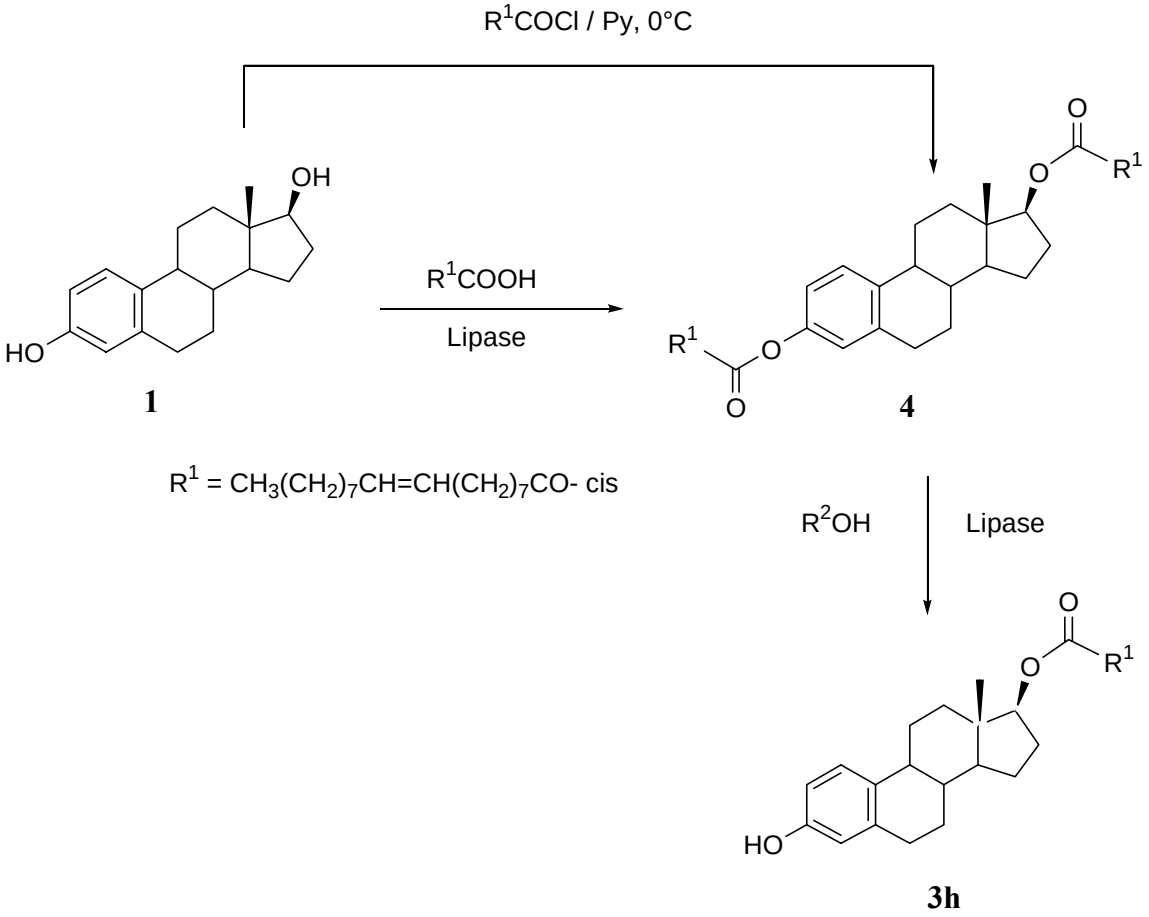
Table 2. Lipase-catalyzed synthesis of 3,17-β-estradiol-17-esters from carboxylic acids (**3**)

Entry	Product	Acylating agent	Yield (%)
1	**3**[a]	Valeric acid	67
2	**3b**	Caproic acid	66
3	**3c**	Capric acid	69
4	**3d**	Lauric acid	73
5	**3e**	Myristic acid	78
6	**3f**	Palmitic acid	70
7	**3g**	Estearic acid	71
8	**3h**	Oleic acid	76
9	**3i**	Elaidic acid	75
10	**3j**	Linoleic acid	72
11	**3k**	Linolenic acid	77

[a] Isolated products. E/S: 5, Acylating/S: 2.5, temperature: 55°C, time 72h.

2. Enzymatic alcoholysis

Another approach to obtain 3,17-β-estradiol-17-oleate **3h** could be an enzymatic alcoholysis on the dioleyl derivative of 3,17-β-estradiol **4** (Scheme 2).



Scheme 2

First, we prepared **4**, following the reported chemical procedure with oleoyl chloride in pyridine at 0°C.[27] This procedure afforded a mixture of products: a major product which is the 3,17 diester and two minor products corresponding to the C-3 monoester and C-17 monoester, as well as free estradiol. The dioleoyl derivative **4** was isolated and purified by column chromatography. When we tested the performance of lipases in the preparation of **4**, we observed that working without solvent at 55°C, CRL afforded the 3,17-dioleate in high purity and yield (92%).

So we scaled up this procedure and then carried out the enzymatic alcoholysis of 3,17-β-estradiol-dioleate **4**. The best enzyme in this case was CAL B. The alcoholysis reaction was conducted with several alcohols as nucleophiles and solvent, leading to similar results with all of them (Table 3).

Table 3. Lipase-catalyzed alcoholysis of 3,17-β-estradiol-dioleate (**4**)

Alcohol	Yield (%)
Ethanol	95
Butanol	94
Octanol	90

[a] Determined by GC analysis. E/S: 5, temperature: 55°C.

Although this enzymatic approach involving two steps: preparation and alcoholysis of estradiol dioleate **4**, gave the products in excellent yield, 92% and 91% isolated products respectively, for preparative purposes the acylation procedure to obtain the 17-monoacyl derivatives of 3,17-β-estradiol seems to be simpler and more convenient.

Conclusions

Following an enzymatic methodology a series of acyl esters of 3,17-β-estradiol has been prepared. The 17-monoacyl products were obtained in a high regioselective way by acylation of 3,17-β-estradiol or by alcoholysis of the corresponding diacyl derivatives. Five (**3b**, **3c**, **3d**, **3e** and **3i**) from the eleven compounds prepared, have not been described before. The influence of various reaction parameters was evaluated to determine the reaction conditions. Among the tested lipases, lipase from *Candida rugosa* appeared to be the most appropriate in the acylation reaction CAL B in the alcoholysis. It was observed that in the acylation, the direct esterification with fatty acids gave better results than transesterification with their ethyl esters. The advantages presented by this methodology such as mild reaction conditions, economy and low environmental impact, make the biocatalysis a convenient way to prepare in a regioselective way, monoacyl derivatives of 3,17-β-estradiol containing the aromatic 3-OH free. Some of these compounds (**3a** and **3h**) are recognized as useful products in pharmaceutical industry and belong to the "long acting" estrogens.

Experimental Section

General Procedures. All solvents and reagents were reagent grade and used without purification. Lipase from *Candida rugosa* (CRL) (905 U/mg solid), pancreatin and type II crude from porcine pancreas (PPL) (190 U/mg protein) were purchased from Sigma Chemical Co.; *Candida antarctica* lipase B (CAL B): Novozym 435 (7400 PLU/g) and Lipozyme RM 1M (LIP) (7800 U/g) were generous gifts of Novozymes Latinoamerica Ltda and Novozymes A/S; *Pseudomonas* lipase: Lipase PS Amano (PSL) (33,200 U/g) was purchased to Amano Pharmaceutical Co. All enzymes were used "straight from the bottle".

Enzymatic reactions were carried out on Innova 4000 digital incubator shaker, New Brunswick Scientific Co. at 33°C and 55°C and 200 rpm. Melting points were measured in a Fisher Johns apparatus and are uncorrected. Enzymatic reactions were followed by TLC on Merck Silica gel 60F-254 aluminium sheets (0.2 mm thickness). For column chromatography Merck Silica gel 60 (60-230 mesh) was used. GC analyses were performed on a Hewlett Packard-5890 gas chromatograph, using HP-17 column (10mx0.53mmx0.20μm). The following temperature program was employed 100°C (1 min)/5°C/min/280°C (5 min). FT-IR spectra were obtained on a Shimadzu FTIR-8300 spectrophotometer.^{1}H-NMR and ^{13}C-NMR spectra were recorded at 500 MHz using Bruker AM-500 spectrometer. Chemical shifts are reported in δ units relative to tetramethylsilane (TMS) set at 0 δ, using $CDCl_3$ as solvent, coupling constants are given in Hz. EI-MS were obtained at 70eV using a TRIO-2 VG Masslab Shimadzu QP-5000 and a Finnigan TSQ70 instrument mass spectrometers.

Enzymatic monoacylation

(17β)-Estra-1,3,5(10)-triene-3,17-diol *cis*-9-octadecenoate (3h). To a solution of **1** (1 g, 3.7 mmol) in toluene (300 ml), 2.6 g (9.25 mmol) of oleic acid and 5 g of CRL were added. The suspension was stirred (200 rpm) at 55º C and the progress of reaction was monitored by TLC (hexane/dichloromethane 1:3)) and GC. After the indicated time, the enzyme was filtered off, the solvent evaporated, and the crude residue purified by silica gel chromatography (hexane: dichloromethane 1:3): 1.50 g (76%) of **3h**. m. p.: 38-41°C. IR $\nu_{máx}$ (cm^{-1}) 3467 (OH), 3010, 2933, 2861 (CH), 1702 (C=O). ^{1}H NMR δ 0.83 (s, 3H), 0.88 (t, *J* = 7.02 Hz, 3H), 1.57-1.23 (m, 28H), 1.67-1.60 (m, 2H), 1.78-1.70 (m, 1H), 1.90-1.84 (m, 2H), 2.02 (dd *J* = 12.3 Hz, 6.5 Hz, 4H), 2.29-2.15 (m, 3H), 2.32 (t, *J* = 7.7 Hz, 2H), 2.85-2.80 (m 2H), 4.70 (dd, *J* = 9.06 Hz, 7.95, 1H), 5,35 (m, 2H), 6.57 (d, *J* = 2.74 Hz, 1H), 6.64 (dd, *J* = 8.44 Hz, 2.75 Hz, 1H), 7.14 (d, *J* = 8.35 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$): 12.15, 14.2, 22.5, 23.3, 25.2, 25.4, 25.7, 26.2, 27.1, 27.4, 27.6, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 34.7, 36.7, 38.5, 43.2, 49.8, 82.5, 112 .7, 115.3, 126.6, 127.4, 127.8, 128.3, 130.4, 131.7, 132.6, 138.2, 153.4, 174.1 ppm. EI-MS: *m/z* (relative intensity): 536 [M^{+}] (17), 281(2), 255 (100), 159 (67), 133 (37), 95 (15), 69 (25), 55 (40), 41 (18).[27]

(17β)-Estra-1,3,5(10)-triene-3,17-diol pentanoate (3a). As described for **3h**, but using pentanoic acid (940 mg) as acylating agent: 877 mg (67 %), m.p.: 146-7 °C (lit. 148 °C)[31];

IR $\nu_{máx}$ (cm^{-1}) 3440 (OH), 3015, 2985, 2885 (CH), 1700 (C=O). ^{1}H-NMR δ 0.83 (s, 3H), 0.93 (t, *J* = 7.42 Hz, 3H) , 1.48-122 (m, 8H)), 1.58-1.49 (m, 1H), 1.69-1.59 (m, 2H) , 1.79-1.66 (m, 2H), 1.90-1.82 (m, 2H), 2.30-2.15 (m, 3H), 2.32 (t, *J* = 7.4 Hz, 2H), 2.81 (m, 2H), 4.70 (dd, *J* = 7.8 Hz, 8.0 Hz, 1H), 6.57 (d, *J* = 2.7 Hz, 1H), 6.63 (dd, *J* = 8.2 Hz, 2.7 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H) ppm . ^{13}C-NMR δ 12.1, 13.7, 22.2, 23.2, 26.1, 27.2, 27.3, 27.6, 29.6, 34.4, 36.9, 38.6, 42.9, 43.7, 49.7, 82.5, 112.7, 115.2, 126.5, 132.5, 138.1, 153.4, 174.1 ppm. EI/MS *m/z* (relative intensity): 356 M^{+} (100), 85 (63), 57 (81), 41 (42).

(17β)-Estra-1,3,5(10)-triene-3,17-diol hexanoate (3b). As described for 3h, but using hexanoic acid (1,08 g) as acylating agent: : 898 mg (66 %), m.p.: 118-121°C; IR $\nu_{máx}$ (cm^{-1}) 3438 (OH),

3018, 2980, 2880 (CH), 1710 (C=O). ^{1}H NMR ($CDCl_3$) δ 0.83 (s, 3H), 0..81 (t, *J* = 7.03 Hz, 3H), 1.40-1.25 (m, 9H) , 1.64-1.60 (m, 4H), 1.77-1.71 (m, 2H), 1.89-1.84 (m, 2H), 2.28-2.17 (m, 3H), 2.31 (t, *J* = 7.85 Hz, 2H), 2.82 (m, 2H), 4.69 (dd, *J* = 7.85 Hz, 9.08 Hz, 1H), 6.56 (d, *J* = 2.69 Hz, 1H), 6.62 (dd, *J* = 8.41 Hz, 2.80 Hz, 1H), 7.14 (d, *J* = 8.30 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ (ppm) : 12.1, 13.9, 22.3, 23.3, 24.8, 26.2, 27.2, 27.6, 29.6, 31.3, 34.6, 36.9, 38.5, 42.9, 43.8, 49.8, 82.5, 112.7, 115.2, 126.5, 132.5, 138.2, 153.4, 174.1 ppm. EI-MS *m/z* (relative intensity): 370[M^+] (100), 254 (13), 225 (14), 172 (22), 159 (28), 146 (26), 133 (22), 99(28), 71 (33) 55 (20), 43(72). HR-MS: 370.5359 ($C_{24}H_{34}O_3^+$; calc. 370.5366).

(17β)-Estra-1,3,5(10)-triene-3,17-diol decanoate (3c). As described for **3h**, but using decanoic acid (1.6g) as acylating agent: : 1.08 g (69 %), m.p.: 94-99°C; IR $\nu_{máx}$ (cm^{-1}) 3450 (OH), 3009, 2989, 2853 (CH), 1701 (C=O). ^{1}H NMR δ 0.76 (s, 3H), 0.84 (t, *J* = 7.05 Hz, 3H), 1.44-1.18 (m, 17H), 1.61-1.54 (m, 4H), 1.71-1.64 (m, 2H), 1.83-1.78 (m, 2H), 2.22-2.11 (m, 3H), 2.25 (t, *J* = 7.65 Hz, 2H), 2.79-2.73 (m, 2H), 4.64 (dd, *J* = 7.89 Hz, 9.09 Hz, 1H), 6.50 (d, *J* = 2.66 Hz, 1H), 6.56 (dd, *J* = 8.40 Hz, 2.83 Hz, 1H), 7.08 (d, *J* = 8.48 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ: 12.1, 14.1, 22.7, 23.3, 25.1, 26.2, 27.1, 27.6, 29.1, 29.3, 29.4, 29.6, 29.7, 31.8, 34.6, 36.9, 38.5, 42.9, 43.8, 49.8, 82.4, 112.6, 115.2, 126.5, 132.6, 138.2, 153.3, 174.1 ppm. EI-MS *m/z* (relative intensity): 426 [M^+] (100), 254 (20), 172 (28), 159 (44), 146 (31), 133 (39), 71 (26), 57 (48) 43 (78). HR-MS: 426.6454 ($C_{28}H_{42}O_3^+$; calc. 426.6450).

(17β)-Estra-1,3,5(10)-triene-3,17-diol dodecanoate (3d). As described for **3h**, but using dodecanoic acid (1.85 g) as acylating agent: : 1.22 g (73 %), m.p.: 70-71°C; IR $\nu_{máx}$ (cm^{-1}) 3460 (OH), 3009, 2989, 2853 (CH), 1701 (C=O). ^{1}H-NMR δ 0.83 (s, 3H), 0.88 (t, *J* = 7.45 Hz, 3H) , 1.48-1.06 (m, 20H)), 1.58-1.49 (m, 1H), 1.66-1.59 (m, 4H), 1.78-1.69 (m, 2H), 1.90-1.82 (m, 2H), 2.20-2.14 (m, 3H), 2.32 (t, *J* = 7.7 Hz, 2H) , 2.82 (m, 2H), 4.70 (dd, *J* = 7.8 Hz, 8.0 Hz, 1H), 6.55 (d, *J* = 2.8 Hz, 1H), 6.63 (dd, *J* = 8.5 Hz, 2.8, 1H), 7.14 (d, *J* = 8.2 Hz, 1H) ppm. ^{13}C-NMR δ 12.1, 14.1, 22.7, 23.2, 25.1 26.2, 27.1, 27.6, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7,31.9, 34.6, 36.9, 38.5, 42.9, 43.8, 49.8, 82.5, 112.7, 115.2, 126.5, 132.5, 138.1, 153.8, 174.2 ppm. EI/MS *m/z* (relative intensity): 454 [M^+] (100), 255 (15), 159 (31), 57 (37), 43 (56). HR-MS: 454.6995 ($C_{30}H_{46}O_3^+$; calc. 454.6992).

(17β)-Estra-1,3,5(10)-triene-3,17-diol tetradecanoate (3e). As described for **3h**, but using tetradecanoic acid (2.1 g) as acylating agent: : 1.38 g (78 %), m.p.: 68-72°C; IR $\nu_{máx}$ (cm^{-1}) 3431 (OH), 3011, 2989, 2863 (CH), 1706 (C=O). ^{1}H NMR δ 0.79 (s, 3H), 0.87 (t, *J* = 7.39 Hz, 3H), 1.35-1.07 (m, 24H), 1.61-1.47 (m, 1H), 1.65-1.57 (m, 4H), 1.74-1.67 (m, 2H), 1.89-1.79 (m, 2H), 2.17-2.15 (m, 3H), 2.23 (t, *J* = 7.65 Hz, 2H), 2.87 (m, 2H), 4.78 (dd, *J* = 7.72 Hz, 8.10 Hz, 1H), 6.55 (d, *J* = 2.72 Hz, 1H), 6.63 (dd, *J* = 8.48 Hz, 2.71, 1H), 7.14 (d, *J* = 8.19 Hz, 1H) ppm. ^{13}C NMR δ 12.1, 14.1, 22.7, 23.3, 24.8, 26.2, 27.1, 27.6, 29.1, 29.2, 29.4, 29.5, 29.6, 29.7, 29.7, 31.3, 34.6, 36.9, 38.5, 42.9, 43.8, 49.8, 82.5, 112.7, 115.2, 126.5, 132.5, 138.2, 153.4, 174.1 ppm. EI-MS *m/z* (relative intensity): 482 [M^+] (52), 255 (13), 172 (14), 159 (38), 146 (24), 133 (23) ,71 (16), 43 (100). HR-MS: 482.7529 ($C_{32}H_{50}O_3^+$; calc. 482.7532).

(17β)-Estra-1,3,5(10)-triene-3,17-diol hexadecanoate (3f). As described for **3h**, but using hexadecanoic acid (2.4 g) as acylating agent: : 1.31 g (70 %), m.p.: 71-73°C. IR $\nu_{máx}$ (cm^{-1}) 3423

(OH), 3015, 2990, 2871 (CH), 1701 (C=O). ^{1}H NMR δ 0.82 (s, 3H), 0.88 (t, *J* = 6.94 Hz, 3H) , 1.55-1.22 (m, 3H), 1.65-1.22 (m, 4H), 1.78-1.70 (m, 1H), 1.90-1.84 (m, 2H), 2.26-2.15 (m, 3H), 2.31 (t, *J* = 7.83 Hz, 3H), 2.85-2.80 (m, 1H), 4.69 (dd, *J* = 7.93 Hz, 9.10 Hz, 1H), 6.56 (d, *J* = 2.67 Hz, 1H), 6.62 (dd, *J* = 8.42 Hz, 2.77 Hz, 1H), 7.14 (d, *J* = 8.32 Hz, 1H) ppm. ^{13}C NMR δ 12.1, 14.2, 23.3, 25.2, 26.2, 27.2, 27.6, 29.2, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 29.7, 31.9, 34.7, 36.9, 38.6, 43.0, 43.8, 49.8, 82.5, 112.7, 115.3, 126.6, 132.6, 138.2, 153.4, 174.1 ppm. EI-MS *m/z* (relative intensity): 510 [M^+] (50), 255 (13), 172 (14), 159 (34), 146 (21), 133 (28) ,71 (16), 43 (100).[27]

(17β)-Estra-1,3,5(10)-triene-3,17-diol octadecanoate (3g). As described for **3h**, but using octadecanoic acid (2.63 g) as acylating agent: : 1.40 mg (71 %), m.p.: 65-67°C. IR $\nu_{máx}$ 3439 (OH), 3011, 2983, 2851 (CH), 1708 (C=O). ^{1}H-NMR δ 0.83 (s, 3H), 0.88 (t, *J* = 7.0 Hz, 3H) , 1.48-1.20 (m, 32H)), 1.65-1.49 (m, 5H), 1.77-1.70 (m, 2H), 1.90-1.81 (m, 2H), 2.28-2.16 (m, 3H), 2.31 (t, *J* = 7.1 Hz, 2H), 2.82 (m, 2H), 4.70 (dd, *J* = 7.6 Hz, 7.9 Hz, 1H), 6.55 (d, *J* = 2.9 Hz, 1H), 6.63 (dd, *J* = 8.3 Hz, 2.9 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 1H) ppm. ^{13}C-NMR δ 12.1, 14.2, 22.7, 23.2, 25.2 26.3, 27.2, 27.6, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 32.0, 34.7, 37.0, 38.6, 43.0, 43.8, 49.8, 82.5, 112.7, 115.3, 126.6, 132.6, 138.2, 153.4, 174.1 ppm. EI/MS *m/z* (relative intensity): 538 [M^+] (100), 255 (35), 159 (60), 57 (53), 43 (78). [27]

(17β)-Estra-1,3,5(10)-triene-3,17-diol *trans*-9-octadecenoate (3i). As described for **3h**, but using trans-9-ocatadecenoic acid (2.6 g) as acylating agent: : 1.48 g (75 %), m.p.: 60-64°C; IR $\nu_{máx}$ (cm^{-1}) 3429 (OH), 3000, 2983, 2850 (CH), 1710 (C=O). ^{1}H NMR δ 0.82 (s, 3H), 0.87 (t, *J* = 6.95, 3H), 1.56-1.23 (m, 28H), 1.65-1.61 (m, 2H), 1.78-1.69 (m, 1H), 1.90-1.83 (m, 2H), 2.01 (dd *J* = 12.3, 6.5 4H), 2.30-2.16 (m, 3H), 2.31 (t, *J* = 7.60 Hz, 2H), 2.85-2.80 (m 2H), 4.70 (dd, *J* = 9.06 Hz, 7.81 Hz, 1H), 5,35 (m, 2H), 6.57 (d, *J* = 2.74 Hz, 1H), 6.65 (dd, *J* = 8.37 Hz, 2.79 Hz, 1H), 7.14 (d, *J* = 8.37 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$): 12.1, 14.2, 22.7, 23.3, 25.1, 25.5, 25.6, 26.3, 27.2, 27.3, 27.6, 29.1, 29.2, 29.4, 29.6, 29.7, 29.7, 31.9, 33.8, 36.9, 38.6, 43.8, 49.8, 82.5, 112.8, 115.3, 126.6, 127.2, 127.6, 128.3, 130.3, 131.0 131.9, 138.2, 153.4, 174.1 ppm. EI-MS *m/z* (relative intensity): 536 [M^+] (20), 255 (100), 159 (74), 133 (37), 95 (13), 69 (21), 55 (43), 41 (29). HR-MS: 536.8450 ($C_{36}H_{56}O_3^+$; calc. 536.8459).

(17β)-Estra-1,3,5(10)-triene-3,17-diol *cis,cis*-9,12-octadecadienoate (3j). As described for **3h**, but using *cis,cis*-9,12-octadecadienoic acid (2.6 g) as acylating agent: 1.41 g (72%). m.p.: 35-37°C. IR $\nu_{máx}$ (cm^{-1}) 3414 (OH), 3005, 2981,2849 (CH), 1703 (C=O). ^{1}H-NMR δ 0.83 (s, 3H), 0.88 (t, *J* = 7.0 Hz, 3H) , 1.48-1.18 (m, 13H)), 1.57-1.39(m, 3H),1.67-1.58 (m, 5H), 1.78-1.69 (m, 2H), 1.90-1.82 (m, 2H), 2.11-1.92(m,4H,) 2.28-2.12 (m, 3H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.1 (m, 4H), 4.70 (dd, *J* = 7.9 Hz, 7.9 Hz, 1H), 5.43-5.27 (m,4H), 6.55 (d, *J* = 2.5 Hz, 1H), 6.63 (dd, *J* = 8.3 Hz, 2.5 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 1H) ppm. ^{13}C-NMR δ 12.1, 14.2, 22.7, 23.3, 25.1, 25.5, 25.6, 26.3, 27.2, 27.3, 27.6, 29.1, 29.2, 29.4, 29.6, 29.7, 29.8, 31.9, 34.6, 36.9, 38.6, 43.8, 49.8, 82.5, 112.8, 115.3, 126.6, 127.2, 127.6, 128.3, 130.3, 132.0 132.5, 138.2, 153.4, 174.1 ppm. EI/MS *m/z* (relative intensity): 532 [M^+] (100), 255 (35), 133 (29), 55 (48), 41 (67). [27]

3,17-β-Estradiol-17-*cis,cis,cis*-9,12,15-octadecatrienoate (3k). As described for **3h**, but using *cis,cis,cis*-9,12,15-octadecatrienoic acid (2.6 g) as acylating agent: 1.51 g (77 %). Oil. IR $\nu_{máx}$

(cm^{-1}) 3433 (OH), 3010, 2980,2846 (CH), 1705 (C=O).^{1}H-NMR δ 0.83 (s, 3H), 0.88 (t, *J* = 7.0 Hz, 3H), 1.48-1.18 (m, 11H)), 1.57-1.39(m, 3H),1.67-1.58 (m, 5H), 1.78-1.69 (m, 2H), 1.90-1.82 (m, 2H), 2.11-1.92(m,4H,) 2.28-2.12 (m, 3H), 2.31 (t, *J* = 7.2 Hz, 2H) , 2.1 (m, 4H), 4.70 (dd, *J* = 7.9 Hz, 7.9 Hz, 1H), 5.43-5.27 (m,6H), 6.55 (d, *J* = 2.5 Hz, 1H), 6.63 (dd, *J* = 8.3 Hz, 2.5 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 1H) ppm. ^{13}C-NMR δ 12.1, 14.2, 22.7, 23.3, 25.1, 25.5, 25.6, 26.3, 27.2, 27.3, 27.6, 29.1, 29.2, 29.4, 29.6, 29.7, 29.8, 31.9, 34.6, 36.9, 38.6, 43.8, 49.8, 82.5, 112.8, 115.3, 126.6, 127.2, 127.6, 128.3, 130.3, 132.0 132.5, 138.2, 153.4, 174.1 ppm. EI/MS *m/z* (relative intensity): 532 [M^{+}] (100), 255 (35), 133 (29), 55 (48), 41 (67).[32]

3,17-β-Estradiol-*cis*-9-dioctadecenoate (4)

Chemical synthesis

It was prepared as described in reference 27 with 10 g of 3,17-β-estradiol. The dioleate **4** was isolated from the mixture of products by column chromatography (hexane/dichloromethane 1:3). Yield: 6.8 g (57%). Oil. IR $\nu_{máx}$ (cm^{-1}) 3007, 2990, 2835 (CH), 1735, 1702 (C=O). ^{1}H-NMR 0.83 (s, 3H), 0.88 (t, *J* = 6.9 Hz, 6H) , 1.58-1.20 (m, 44H)), 1.67-1.60 (m, 6H), 1.78-1.70 (m, 2H), 1.90-1.82 (m, 2H), 2.11-1.95(m, 8H,) 2.28-2.20 (m, 3H), 2.31 (t, *J* = 7.0 Hz, 4H) , 2.89-2.83 (m, 2H). 4.70 (dd, *J* = 7.9 Hz, 7.9 Hz, 1H), 5.39-5.30 (m, 4H), 6.78 (d, *J* = 2.5 Hz, 1H), 6.82 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H), 7.27 (d, *J* = 8.5 Hz, 1H) ppm.^{13}C-NMR δ 12.1, 14.1, 21.1, 22.7, 23.2, 25.0, 25.1, 26.1, 27.0, 27.1, 27.2, 27.6, 29.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 31.9, 34.4, 34.6, 36.9, 38.2, 43.0, 44.0, 49.8, 82.5, 118.6, 121.4, 126.3, 129.6, 129.7, 129.9, 130.0, 137.7, 138.1, 148.5, 172.5, 173.9 ppm. EI/MS *m/z* (relative intensity): 535 (25), 279 (15), 133 (29), 55 (100), 41 (43).

Enzymatic synthesis

To a solution of **1** (1 g, 3.7 mmol), 9.8 g (34.5 mmol) of oleic acid and 5 g of CRL were added. The suspension was stirred (200 rpm) at 55º C and the progress of reaction was monitored by TLC (hexane/dichloromethane 1:3) and GC. After 72 h, 100 ml of dichloromethane were added and the enzyme was filtered off. The solvent was evaporated, and the crude residue was purified by silica gel chromatography (hexane/dichloromethane 1:3): 2.7 g (92%) of **4**. Spectroscopic data were in accordance with **4** obtained by chemical synthesis.

Enzymatic alcoholysis of 3,17-β-estradiol-*cis*-9-dioctadecenoate (4)

To a solution of **4** (500 mg (0.62 mmol)) in 10 ml of alcohol, 2.5 g of CAL B were added. The suspension was shaken (200 rpm) at 55°C and the progress of reaction was monitored by GC. After 72 h, the enzyme was filtered off, the solvent evaporated, and the crude residue was purified by silica gel chromatography (hexane:dichloromethane 1:3): 316 mg (95%) of **3h** Spectroscopic data were in accordance with **3h** obtained by enzymatic acylation of **1**.

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